The role of peripheral T-cell deletion in transplantation tolerance

Andrew D. Wells¹, Xian-Chang Li², Terry B. Strom² and Laurence A. Turka^{1*}

¹Department of Medicine, University of Pennsylvania, 415 Curie Boulevard, Philadelphia, PA 19104, USA

²Department of Medicine, Harvard Medical School, Division of Immunology, Beth Israel Deaconess Medical Center, Boston, MA 02215, USA

The apoptotic deletion of thymocytes that express self-reactive antigen receptors is the basis of central (thymic) self-tolerance. However, it is clear that some autoreactive T cells escape deletion in the thymus and exist as mature lymphocytes in the periphery. Therefore, peripheral mechanisms of tolerance are also crucial, and failure of these peripheral mechanisms leads to autoimmunity. Clonal deletion, clonal anergy and immunoregulation and/or suppression have been suggested as mechanisms by which 'inappropriate' T-lymphocyte responses may be controlled in the periphery. Peripheral clonal deletion, which involves the apoptotic elimination of lymphocytes, is critical for T-cell homeostasis during normal immune responses, and is recognized as an important process by which self-tolerance is maintained. Transplantation of foreign tissue into an adult host represents a special case of 'inappropriate' T-cell reactivity that is subject to the same central and peripheral tolerance mechanisms that control reactivity against self. In this case, the unusually high frequency of naive T cells able to recognize and respond against non-self-allogeneic major histocompatibility complex (MHC) antigens leads to an exceptionally large pool of pathogenic effector lymphocytes that must be controlled if graft rejection is to be avoided. A great deal of effort has been directed toward understanding the role of clonal anergy and/or active immunoregulation in the induction of peripheral transplantation tolerance but, until recently, relatively little progress had been made towards defining the potential contribution of clonal deletion. Here, we outline recent data that define a clear requirement for deletion in the induction of peripheral transplantation tolerance across MHC barriers, and discuss the potential implications of these results in the context of current treatment modalities used in the clinical transplantation setting.

Keywords: peripheral deletion; apoptosis; T lymphocyte; interleukin 2; tolerance

1. 'ACTIVE' VERSUS 'PASSIVE' CELL DEATH IN PERIPHERAL T-LYMPHOCYTE HOMEOSTASIS

Two distinct forms of apoptotic cell death can lead to the elimination of activated T cells (Van Parijs *et al.* 1996). 'Passive' cell death occurs when activated T cells are deprived of growth factors, and can be blocked by the anti-apoptotic proteins Bcl-2 and Bcl-xL (Huang *et al.* 1999; Van Parijs *et al.* 1996). Blockade of T-cell co-stimulatory signals, a strategy that has been used successfully to induce tolerance in experimental transplant models (Sayegh & Turka 1998; Van Parijs *et al.* 1996), results in impaired expression of Bcl-xL and the T-cell growth factor interleukin 2 (IL-2), and therefore promotes passive cell death. 'Active' cell death tends to occur in CD4⁺ T cells that receive repeated stimulation through their antigen receptors, is relatively independent of CD28 co-stimulation, and is mediated by signals transduced through the tumour necrosis factor receptor (TNFR) family member Fas/CD95 (Van Parijs & Abbas 1998). Unlike passive cell death, this form of apoptosis requires IL-2 and occurs despite the expression of Bcl-2 or Bcl-xL. Active cell death occurs similarly in the CD8⁺ T-cell

subset, but can be mediated by signals from either Fas or the TNFR (Zheng *et al.* 1995).

2. CLONAL DELETION AS A MECHANISM OF PERIPHERAL SELF-TOLERANCE

Passive cell death due to growth factor deprivation is normally associated with the decline in antigen-specific T-cell number following an acute phase of clonal expansion, such as that which occurs during an acute viral infection. This homeostatic downregulation of the T-lymphocyte response requires neither Fas- nor TNFR-mediated signals (Lohman *et al.* 1996; Reich *et al.* 2000). Conversely, active T-cell death appears to be especially important in the maintenance of self-tolerance in the periphery, as mice that are deficient in the production of IL-2, or in the expression of either Fas or its ligand, FasL/CD95L, accumulate activated, autoreactive T cells in their peripheral organs and suffer autoimmune pathology as a consequence (Van Parijs & Abbas 1998). The importance of active peripheral deletion in the control of autoreactive T lymphocytes has been further demonstrated in an elegant neo-self-antigen model of diabetes. In this model, major histocompatibility complex (MHC) class I-restricted presentation of pancreatic β -cell autoantigen by

*Author for correspondence (turka@mail.med.upenn.edu).

circulating antigen-presenting cells leads to the activation, proliferation and Fas-mediated deletion of autoreactive CD8⁺ T cells residing in draining lymphoid tissue (Kurts *et al.* 1997b). If the deletion of the autoreactive CD8⁺ T cells is inhibited, autoimmune diabetes ensues (Kurts *et al.* 1997a). Peripheral tolerance induced by oral feeding of antigen (Marth *et al.* 1999), or by viral (Jones *et al.* 1990) and bacterial (Kawabe & Ochi 1991) super-antigens also involves the active Fas-mediated deletion of antigen-reactive T cells (Bonfoco *et al.* 1998).

Several recent studies have shown that passive cell death is also operative in the maintenance of self-tolerance. Immune-mediated pathology in experimental autoimmune encephalitis, a model of the autoimmune demyelinating disease multiple sclerosis, is controlled primarily through the Fas-independent, passive apoptosis of autoreactive T cells in the central nervous system (Dittel *et al.* 1999; Issazadeh *et al.* 2000). Also, lymphocytes from mice deficient for the pro-apoptotic bcl-2 family member Bim are resistant to apoptosis mediated by cytokine withdrawal, but not to Fas-induced cell death (Bouillet *et al.* 1999). Interestingly, Bim-deficient animals progressively develop severe autoimmune disease. These data suggest that growth factor deprivation may contribute to T-cell homeostasis during both acute and chronic immune responses.

3. CLONAL DELETION IS REQUIRED FOR THE INDUCTION OF PERIPHERAL TRANSPLANTATION TOLERANCE

T-cell apoptosis has been associated with tolerance induction in a number of transplantation models, and much interest has been directed recently at a potential role for active, Fas-mediated cell death in the induction of transplantation tolerance (Kabelitz 1998). Some of this interest was sparked by the realization that naturally 'immune-privileged' tissues such as the eye and testis constitutively express FasL, and apparently inhibit immune inflammatory responses by actively deleting infiltrating, Fas-expressing T lymphocytes (Griffith *et al.* 1995; Streilein 1993). This concept has been subsequently applied to the transplantation setting, using muscle cells engineered to express FasL to protect co-engrafted, allogeneic pancreatic islets from immune-mediated rejection (Lau *et al.* 1996). This approach has been difficult to replicate, and has met with significant biological hurdles, because ectopic expression of FasL in many tissues has more recently been shown to have a distinct pro-inflammatory effect, leading to accelerated destruction of the graft instead of prolonged survival (Allison *et al.* 1997; Chen *et al.* 1998; Kang *et al.* 1997). This complication has been avoided to some extent by pretreating recipients of allogeneic organ transplants with donor-specific dendritic cells engineered to express FasL (Min *et al.* 2000). This strategy induces hyporesponsiveness to alloantigen *in vivo*, and results in prolonged acceptance of subsequently transplanted donor organs.

Peripheral deletion of donor-reactive T cells has been linked to tolerance induced by co-stimulatory blockade during allogeneic bone marrow transplantation (Wekerle *et al.* 1998). In this model, recipients of allogeneic bone marrow are subjected to cytoreductive therapy, such as whole body irradiation, in order to reduce the number of

potentially donor-reactive lymphocytes in the periphery. Long-term engraftment of allogeneic donor bone-marrow-derived cells within the host haematopoietic compartment (chimerism) is observed, and robust donor-specific tolerance is subsequently achieved by central deletion of donor-reactive T cells in the thymus. However, long-term engraftment of allogeneic stem cells can also be achieved in this model without overt lymphoablative conditioning of the recipient, using high doses of donor bone marrow administered under conditions of combined CD28 and CD154 co-stimulatory blockade (Wekerle *et al.* 2000). Under these conditions, donor stem-cell engraftment is preceded by the disappearance of donor-reactive T cells in the periphery of the recipient, is dependent on allogeneic bone marrow transfer, and is only observed during treatment modalities that lead to long-term donor-specific tolerance. These data suggest that co-stimulatory blockade in this model functions in part to induce the elimination of donor-reactive T cells in the periphery, and that this phase of clonal deletion is important for the prevention of acute rejection of allogeneic bone marrow, allowing for eventual donor-specific tolerance.

A role for growth factor deprivation-induced, passive T-cell apoptosis in the induction of solid organ transplantation tolerance by co-stimulatory blockade has also been recently defined (Bertolino *et al.* 1995; Wells *et al.* 1999). Co-stimulatory signals function during T-cell activation in the upregulation of growth factor and cytokine gene expression (Janeway & Bottomly 1994), as well as in the induction of survival-associated genes such as *bcl-xL* (Boise *et al.* 1995). For instance, the *in vitro* stimulation of alloreactive T cells with allogeneic hepatocytes, which lack B7 molecules and therefore cannot provide CD28 co-stimulatory signals, results in impaired production of IL-2, abortive proliferation, failure to induce anti-apoptotic *bcl* gene expression, and premature, Fas-independent T-cell apoptosis (Bertolino *et al.* 1995). Therefore, transplantation tolerance induced by the blockade of co-stimulatory signals could be due to the reduced proliferation, impaired differentiation or anergy of alloreactive T cells, or to the increased susceptibility of alloreactive T cells to passive cell death. These two general downstream effects of co-stimulatory blockade have been successfully uncoupled using mice that constitutively express Bcl-xL within the T-cell lineage (Grillot *et al.* 1995). Wild-type T cells and Bcl-xL-transgenic T cells are equally susceptible to the induction of clonal anergy and impairment of effector function following antigenic stimulation in the absence of co-stimulation. However, Bcl-xL-transgenic T cells are remarkably resistant to passive apoptosis induced by co-stimulatory blockade, and can persist *in vivo* in numbers exceeding those of wild-type T cells by 100- to 1000-fold for several months (Wells *et al.* 1999). This increased resistance to passive cell death translates to resistance to tolerance induction, as Bcl-xL-transgenic mice reject MHC-mismatched allografts under conditions of co-stimulatory blockade that induce long-term allograft survival and donor-specific tolerance in wild-type recipients (Wells *et al.* 1999). These results suggest that passive cell death is an important component of the tolerance-inducing effects of co-stimulatory blockade.

Recent studies have also implicated a role for active cell death in the induction of peripheral transplantation

tolerance. For example, IL-2 is required for the induction of tolerance to MHC-mismatched allografts (Dai *et al.* 1998; Wells *et al.* 1999). IL-2 serves two distinct and opposing functions for T cells: as a growth factor, and as a death factor. The activity of IL-2 as a growth factor is highly redundant, as several other interleukins (IL-4, IL-7, IL-9 and IL-15) that signal through the IL-2 receptor common gamma chain (γ_c) can also serve as growth factors for T cells (Sugamura *et al.* 1996). This redundancy is apparent in mice deficient in IL-2, which are immunocompetent (Kundig *et al.* 1993; Schorle *et al.* 1991), exhibit efficient cellular proliferation *in vivo* (Khoruts *et al.* 1998) and can reject allogeneic organ transplants (Steiger *et al.* 1995). However, IL-2 is uniquely required for the induction of active apoptosis in T cells, as other T-cell growth factors cannot substitute for IL-2 in the mediation of this negative regulatory activity (Van Parijs & Abbas 1998). For instance, significant numbers of apoptotic T cells can be detected in wild-type mice that are rejecting allogeneic cardiac transplants, but apoptotic cells are essentially undetectable in IL-2-deficient recipients that are undergoing graft rejection (Dai *et al.* 1998). In normal transplant recipients tolerized by co-stimulatory blockade, the number of apoptotic T cells is significantly increased (Dai *et al.* 1998; Li *et al.* 1999b). However, IL-2-deficient transplant recipients subjected to co-stimulatory blockade remain relatively devoid of apoptotic T cells, and tolerance induction fails in these mice (Dai *et al.* 1998). The role of IL-2 in the induction of transplantation tolerance has been further dissected in a separate model using the immunosuppressive drug rapamycin. Rapamycin inhibits the use of IL-2 by antagonizing a discrete biochemical step in IL-2 receptor-coupled signal transduction (Nourse *et al.* 1994; Price *et al.* 1992). In normal mice, rapamycin treatment during transplantation results in impaired growth-factor-driven T-cell proliferation (Bierer *et al.* 1990; Dumont *et al.* 1990), but IL-2-mediated T-cell death occurs normally (Wells *et al.* 1999) and long-term allograft survival and donor-specific tolerance is achieved (Chen *et al.* 1994). Conversely, in IL-2-deficient mice, which are defective in active T-cell apoptosis, rapamycin treatment during transplantation fails to induce long-term allograft survival and tolerance (Wells *et al.* 1999). The combination of rapamycin and normal versus IL-2-deficient mice in this transplant model successfully dissociates the growth-promoting effects of IL-2 from its death-promoting effects, and the results of this study demonstrate that the basis for the requirement of IL-2 in the induction of transplantation tolerance lies in the death-promoting activity of this cytokine. Together, the studies described above clearly demonstrate that peripheral deletion is absolutely required for the induction of tolerance across MHC barriers.

4. MECHANISMS OF T-CELL APOPTOSIS OPERATIVE DURING TRANSPLANTATION TOLERANCE

The studies discussed above show that both active and passive deletional mechanisms play a significant role in the induction of transplantation tolerance. The abundance of data describing the biochemical events that mediate active versus passive cell death allows us to make certain

predictions as to which signal transduction pathways might control the deletion of alloreactive T cells during the induction of transplantation tolerance. As discussed above, IL-2 is required for both Fas-mediated apoptosis in activated T cells, and for the induction of transplantation tolerance. These facts might easily lead one to conclude that Fas-mediated signal transduction is necessary for the induction of tolerance to allografts. However, although Fas-mediated signals may be operative during an alloimmune response (Kabelitz 1998), Fas is not required for the induction of transplantation tolerance (Li *et al.* 1999a), nor is it required for either the peripheral deletion of alloreactive T cells *in vivo* (Li *et al.* 1999a) or the IL-2-driven apoptosis of T cells stimulated *in vitro* in the absence of CD28 co-stimulation (Bertolino *et al.* 1995; Wells *et al.* 1999) (A. D. Wells, Xian-Chang Li, T. B. Strom & L. A. Turka, unpublished data). This suggests that an IL-2-dependent mode of cell death exists that does not operate through Fas, and is required for the induction of transplantation tolerance. This would seem to oppose the current view that IL-2-dependent 'activation-induced' cell death (AICD) is mediated exclusively by Fas–FasL interactions, and may point to a role for other death-inducing members of the TNFR family in this form of cell death. Alternatively, this apparent contradiction can be resolved by broadening the concept of AICD to include passive cell death associated with growth factor deprivation. For instance, recent studies suggest that 'passive' cell death is not as passive as we once thought. The susceptibility of activated T cells to passive cell death *in vitro* and *in vivo* increases with each round of cell division, and can be blocked by cyclosporin A, an immunosuppressive drug that blocks early T-cell activation (Li *et al.* 1999b). Although the T cells in this model die later in the response due to the cessation of growth-factor-mediated signals, the sensitivity of these cells to this mode of apoptosis is dependent on their previous activation and growth-factor-driven cell division. A potential biochemical basis for the activation-dependent nature of passive cell death is the requirement for mitogenic signalling through Ras in the induction of protein phosphatase 1 α -mediated activation of Bad, a pro-apoptotic bcl-2 family member involved in growth factor deprivation-induced apoptosis in T cells (Ayllon *et al.* 2000). Similarly, T cells deficient in a separate pro-apoptotic bcl-2 family member, Bim, are resistant to apoptosis induced by growth factor deprivation, but remain susceptible to Fas-mediated apoptotic signals (Bouillet *et al.* 1999). This suggests that a distinct apoptotic programme is initiated specifically in response to biochemical cues associated with growth factor deprivation. The bcl-2 family of pro- and anti-apoptotic proteins functions to a large degree in the perturbation versus homeostasis of mitochondrial membrane potential (Rathmell & Thompson 1999). Interestingly, a recent study has demonstrated a role for reactive oxygen intermediates in the breakdown of mitochondrial membrane potential and induction of Fas-independent apoptosis in activated T cells subjected to growth factor deprivation (Hildeman *et al.* 1999). These studies suggest that regulation of Bcl-2-related proteins and mitochondrial oxidative phosphorylation during T-cell activation and proliferation may influence the susceptibility of T cells to apoptosis upon subsequent deprivation of growth factor later in the

response. T-cell apoptosis induced by growth factor withdrawal therefore depends on active signal transduction and involves the active execution of cell death programmes by pro-apoptotic mediators and, in this respect, we suggest that this mode of cell death clearly qualifies as 'activation-induced' cell death.

Together, these data suggest that active, Fas/TNFR-mediated apoptosis and passive, growth factor deprivation-induced apoptosis may be more accurately thought of as two specific cases of AICD: one that is the immediate result of signals transduced to activated T cells through 'death' receptors, and one that results from the withdrawal of survival signals from activated T cells. In this way, peripheral T-cell deletion during transplantation tolerance can be seen to result from distinct apoptotic signals that lead to a broadly operative process of activation-induced cell death.

5. HOW DOES CLONAL DELETION FACILITATE THE INDUCTION OF TRANSPLANTATION TOLERANCE?

We have proposed a relatively simple model in which the size of the reactive T-cell pool determines the relative importance of peripheral deletion in the induction of tolerance. According to this hypothesis, the exceptionally high frequency of naive T cells able to directly recognize allogeneic MHC antigens (as many as 1 in 10 naive T cells) leads to the formation of an alloreactive effector pool so large that a significant reduction in the number of alloreactive T cells must occur in order for any tolerance-inducing protocol to achieve its goal. In this model, co-stimulatory blockade serves not only to impair the generation of potentially pathogenic effector cells from the allospecific naive T-cell pool, but also serves to reduce the survival capacity of the responding cells. In this way, an alloimmune response occurring under conditions of co-stimulatory blockade results in a relatively small pool of surviving allospecific T cells, within which the frequency of cells with the potential to effect graft rejection is very small (Wells *et al.* 1999). However, if alloreactive T-cell deletion is impaired under these same conditions, the sheer number of T cells reactive against allogeneic MHC antigens ensures that enough effector cells survive to effect graft rejection. A similar phenomenon may be responsible for the failure of immune deviation to induce tolerance across MHC barriers. Acute graft rejection is associated with IL-2- and interferon (IFN)- γ -producing (Th1) effector cells in the graft, while most of the functional T cells that remain after transplantation under conditions of co-stimulatory blockade (and other protocols that prevent rejection) are of the Th2 phenotype (Sayegh *et al.* 1995). These results have suggested that overt deviation of the alloimmune response from Th1 toward Th2 may result in prolonged allograft survival. However, this approach fails to induce tolerance to major-mismatched allografts in the absence of other tolerance-inducing protocols (Li *et al.* 1998a). Although the frequency of Th1-effector cells is much lower under Th2-polarizing conditions, it is likely that, again, the sheer number of T cells reactive against allogeneic MHC antigens ensures that enough Th1 cells are always present to effect graft rejection.

An important prediction of our 'pool size' model is that peripheral deletion may be less important for the induction

of tolerance to antigens against which the reactive T-cell frequency is relatively small. Indeed, this prediction has so far held true. For instance, immune deviation alone can induce prolonged survival of minor-mismatched allografts (Li *et al.* 1998a), a situation in which the size of the reactive T-cell pool is relatively small (between 1 in 1000 and 1 in 100; A. D. Wells, Xian-Chang Li, T. B. Strom and L. A. Turka, unpublished data). Also, Bcl-xL-transgenic mice, which are resistant to tolerance induction against major-mismatched allografts, can be tolerized against minor histocompatibility complex antigens (A. D. Wells, Xian-Chang Li, T. B. Strom and L. A. Turka, unpublished data). In the cases above, the size of the minor antigen-reactive T-cell pool is apparently small enough to be controlled by immune deviation, clonal anergy or suppression, without the need for further elimination of alloreactive T cells via clonal deletion. These data also indicate that, although peripheral deletion appears to be necessary for the prolonged survival of major-mismatched allografts, it is most likely not sufficient for the maintenance of long-term, donor-specific tolerance. Indeed, clonal anergy induced by co-stimulatory blockade appears to function in the short-term inhibition of acute rejection. However, clonal anergy is susceptible to reversal by IL-2 (Beverly *et al.* 1992; Sayegh *et al.* 1995; Tran *et al.* 1997) and may 'wear off' over time (Pape *et al.* 1998; Wells *et al.* 1999). Therefore, induction of an active immunoregulatory state is the most likely basis of long-term transplantation tolerance (Waldmann 1999).

While the role of peripheral clonal deletion in the control of chronic rejection and maintenance of tolerance long after the initial transplant is unclear, recent data suggest that peripheral deletion may facilitate the development of active suppression early in the alloimmune response. During an inflammatory response, circulating, immature dendritic cells (DCs) that capture antigens from necrotic or apoptotic cell debris are induced to mature into potent antigen-presenting cells for T-cell activation. In the absence of inflammation, however, apoptotic cells do not induce the maturation of DCs, so any antigen captured during the process is not effectively presented to T cells (Gallucci *et al.* 1999; Steinman *et al.* 2000). Furthermore, apoptotic cells can also have direct tolerogenic effects *in vivo*. T cells induced to undergo active apoptosis within immune-privileged sites have been shown to produce IL-10 immediately before their death, and DCs that engulf these cells gain the ability to tolerize live antigen-reactive T cells that they encounter subsequently (Gao *et al.* 1998; Griffith *et al.* 1996). These data suggest that apoptotic T cells generated during co-stimulatory blockade may inhibit the maturation and function of local antigen-presenting cells, and may also create an environment that favours the development of regulatory T cells.

Apoptotic death and production of IL-10 by T cells may also cooperate with specific modes of co-stimulatory blockade in the induction of allospecific regulatory T-cell populations. Blockade of CD40-CD40L interactions using antibodies that bind to CD40L on the T cell has proven to be a successful mode of tolerance induction in multiple transplantation models (Sayegh & Turka 1998). Although much of the effects of CD40-CD40L blockade can be attributed to the inhibition of CD40-mediated

activation of antigen-presenting cells, it is clear that the anti-CD40L reagents used in both murine and human models also transduce signals to the T cells. A recent study has investigated the specific effects of CD40L signals on the fate of T cells during CD40–CD40L blockade (Blair *et al.* 2000). Co-ligation of TCR and CD40L on T cells can support an abbreviated phase of CD28-independent clonal expansion; however, because this mode of stimulation does not upregulate Bcl-xL, these T cells undergo premature apoptotic deletion. Furthermore, T cells that remain after the deletion phase produce high levels of the suppressive cytokine IL-10, suggesting that these cells may have regulatory capacity (Blair *et al.* 2000). This effect of CD40L ligation on T cells may be relevant during CD28 co-stimulatory blockade as well. Although blockade of CD28 co-stimulation *in vivo* results in greatly impaired T-cell clonal expansion, some proliferation does occur (Gudmundsdottir *et al.* 1999), and this is probably driven by alternative co-stimulatory pathways such as CD40–CD40L. In this situation, T cells responding to alloantigen under CD28 co-stimulatory blockade would suffer from anergy and decreased survival capacity due to the lack of CD28 signals, and would also be induced to differentiate into regulatory cells due to CD40L-mediated induction of IL-10 secretion.

6. T-CELL DELETION, IMMUNOSUPPRESSION AND TOLERANCE: CLINICAL IMPLICATIONS

Clinical success in controlling acute rejection has increased drastically over the past two decades, primarily through the implementation of improved immunosuppressive drugs. However, the increase in short-term graft survival has not translated to lower incidence of chronic rejection (Cecka 1996), suggesting that we are no closer to our goal of inducing long-term graft tolerance in the clinic than we were 20 years ago. Modern immunosuppressive therapy relies heavily on calcineurin inhibitors such as cyclosporin A and FK-506, which block alloimmune responses by directly inhibiting T-cell activation (Bierer *et al.* 1990; Dumont *et al.* 1990). However, both cyclosporin A and FK-506 have been shown to interfere with tolerance induction in a number of small and large animal transplantation models (Kirk *et al.* 1999; Larsen *et al.* 1996; Li *et al.* 1999b, 1998b). Indeed, by interfering with activation-induced apoptosis (Li *et al.* 1999b), calcineurin inhibitors may actually protect alloreactive T cells from clonal deletion, thereby leaving a large population of pathogenic T cells 'waiting in the wings' and available for the mediation of chronic rejection upon discontinuation of immunosuppressive therapy. Alternative immunosuppressive agents may need to be considered, such as rapamycin, which inhibits neither the initial activation nor subsequent deletion of alloreactive T cells (Bierer *et al.* 1990; Dumont *et al.* 1990; Wells *et al.* 1999), and synergizes with co-stimulatory blockade in the induction of long-term, donor-specific transplantation tolerance in rodent models (Li *et al.* 1999b, 1998b).

Current available data suggest that the induction of efficient peripheral T-cell clonal deletion is a crucial component of any attempt to induce tolerance against MHC-mismatched tissues that is not directly lymphoablative. Furthermore, the use of current immunosuppressive

agents, which has led to great success in the clinical management of acute graft rejection, may actually hinder the development of clinically relevant, long-term tolerance to transplanted tissue.

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